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# **Establishment of an Integrated Confocal Microscopy Facility for ONR Biofouling/Biocorrosion Researchers**

## **Final Report Navy N00014-96-1-0093**

The Leica scanning-laser confocal microscope was installed at CEB in late September 1996. A brief training session by a Leica representative was held shortly thereafter. The instrument functions perfectly aside from some non-critical software bugs. It is equipped with three lasers (Kr, Ar, and HeNe) that are fiber-optically combined and delivered into the scan head. The galvanometer-driven stage functions flawlessly even with rather heavy samples (30 g). The following lenses are available: 100X adjustable NA plan apo, 100X 1.3 NA oil-immersion phase contrast, 63X adjustable NA long-working-distance, 40X 1.0 NA oil-immersion phase contrast, 10X 0.3 NA, 60X 0.9 NA water-immersion phase contrast, and 40X 0.75 NA water-immersion phase contrast. Upright and inverted platforms have been used and are functioning well. A Syquest EZ 135 drive (removable hard medium) has been installed and performs well as a data-transfer device. The computer on which the data resides has been established as FTP site and data transfer in this manner has been performed within the UT network. FTP transfer outside the network has not yet been tested.

Initial examination of corrosion samples (mixed-species biofilms on stainless steel substrata) has gone very well, and we are able to reconstruct in three dimensions the microbial biofilm (stained with fluorescent dyes) and visualize its relationship to the substratum and to sulfide granules (using reflectance scanning). This work leads us to believe that simultaneous localization of the microbial biofilm and corrosion pits will be accomplished.

We have mounted the Hamamatsu photon-counting camera on the leica upright platform. This microscope gives superior light throughput to the Zeiss platform on which the camera was originally mounted. We expect to be able to detect lower levels of bacterial bioluminescence (or genetically engineered luciferase activity) using this microscope configuration than was possible with the previous Zeiss-based instrument. Furthermore, the superb resolution in the Z-axis will permit us to resolve in three dimensions the source of bioluminescence within thick biofilms, perhaps at the level of a single bacterial cell.